# Membrane Dynamics & Bilayer Probes II

**3403-Pos Board B508**
**Raft-Like Phase Coexistence Revealed by Solid-State Carbon-13 Separated Local Field Mas NMR**
**Avigdor Leftin**, Klaus Beyer, Michael F. Brown.
Molecular interactions such as hydrogen-bonding and hydrophobic matching are observed for raft-like membranes using solid-state NMR [1]. We applied natural abundance separated-local field magic angle spinning $^{13}C$ NMR to study the components of a canonical raft-like membrane system. This method probes lipid structure at the headgroup, glycerol backbone, and acyl chains simultaneously for each membrane component. We resolved isotropic $^{13}C$ NMR chemical shifts and $^{13}C$-$^{1}H$ residual dipolar couplings (RDCs) for single-component, binary, and ternary membranes. These measurements are interpreted using a mean-torque model providing cross-sectional areas per lipid and volumetric hydrocarbon thicknesses [2]. The structural changes are related to mixing or de-mixing of lipids and cholesterol. In the single-component membranes, EYSM is highly ordered from hydrogen-bonding at the backbone, while POPC is highly disordered due to acyl chain unsaturation. The combination of these lipids leads to a reduction of EYSM order and an increase in POPC order due to inter-chain contacts. Addition of cholesterol yields a significant increase in hydrophobic matching in POPC and EYSM membranes [3]. From these structural results for single-component and binary systems, we evaluated the interactions contributing to lipid mixing in the ternary membrane system. EYSM exhibits a cross-sectional area and hydrocarbon thickness resembling the single-component system. In contrast, POPC exhibits a cross-sectional area and hydrocarbon thickness characteristic of the binary lipid-cholesterol mixture. This suggests a liquid-ordered phase is present in an ideally mixed ternary system. Our experimental results contribute to our understanding of raft-like membrane self-assembly, and can be used to interpret changes in membrane structure that occur during protein recognition events and membrane fusion.
[1] T. Bartels *et al*. (2008) *JACS* **130** 14521-14532. [2] H.I. Petrache *et al*. (2000) *Biophys. J.* **79**, 3172-3192. [3] G.M. Martinez *et al*. (2004) *Langmuir* **20** 1043-1046.

**3404-Pos Board B509**
**First Observation of Dynamics in Lipid Multilayers using X-ray Photon Correlation Spectroscopy (XPCS)**
**Yicong Ma**, Gang Chen, Curt DeCaro, Justin Berry, Mark Servantes, Lobat Tayebi, Zhang Jiang, Suresh Narayanan, Alec Sandy, Hyunjung Kim, Atul Parikh, Laurence Lurio, Sunil Sinha.
Collective modes of layer undulations in lipid multilayers are of considerable interest because they can be used to measure the elastic moduli and viscosity of the lipid bilayers as a function of interlamellar spacing, properties of the interlayer aqueous channels, and temperature. These are fundamental quantities required to calculate the configurations and fluctuations of lipid membranes, relevant in modeling many biomembrane functionalities (e.g., intermembrane interactions and polyvalent ligand recognition) that depend on elasticity and dynamics of membrane phases. However, only relatively few investigations have been made of lipid systems, with e.g. dynamical light scattering, neutron spin echo and inelastic neutron scattering. There remains a gap in time scales and length scales which the technique of x-ray photon correlation spectroscopy (XPCS) can fill.
We present here the first XPCS measurements of the dynamics of 1,2-dioleoyl-sn-glycero-3-phtosphocholine (DOPC) multilayers at relative humidity of 99% and temperature of 28°C. The measurements were done at and near the 1st Bragg peak of the multilayer, in which range the intensity-intensity autocorrelation function includes heterodyne oscillations [1] due to a large static component as well as homodyne oscillations predominated at q-values off the Bragg peak. According to de Jeu's theory [2], there are two different modes of relaxation time in the system: a slow and a fast one. Our experiment reveals the existence of the slow mode, which exhibits a plateau in relaxation time over a range of q (10e-5 Å-1~10e-4 Å-1). The results of the analysis of these correlation functions according to the model of de Jeu et al. will be presented.
1. C. Gutt et al, Phys. Rev. Lett. 91, 076104 (2003)
2. W. H. de Jue et al, Rev. Mod. Phys., 75, 181-235 (2003)

**3405-Pos Board B510**
**Organization and Dynamics of Cholesterol Crystalline Domains using EPR Spin-Labeling**
**Laxman Mainali**, Marija Raguz, Witold K. Subczynski.
EPR spin-labeling methods were used to study the organization and dynamics of cholesterol molecules in membranes formed from Chol/PL (cholesterol/phospholipid) mixtures. Membranes made from phospholipids with different cholesterol solubility thresholds (CST) were investigated. It was confirmed using the EPR discrimination methods that cholesterol crystalline domains (CCDs) were present in all of the membrane suspensions when the mixing ratio exceeded the CST. The behavior of phospholipids was monitored with phospholipid analogue spin labels (n-PCs), and cholesterol with the cholesterol analogue spin labels CSL and ASL. Results indicated that phospholipid and cholesterol mixtures can form a membrane suspension up to a mixing ratio exceeding significantly the CST. EPR spectra for n-PC indicated that phospholipids exist in these suspensions in the lipid-bilayer-like structures. Spectral characteristics of n-PCs (spin labels located outside the CCD) change with increase in the cholesterol content up to and beyond the CST. These results present strong evidence that the CCD forms an integral part of the phospholipid bilayer when formed from Chol/PL mixture up to a mixing ratio of 2 to 3. EPR spectra for CSL and ASL in the CCD and the PCD were very similar indicating that in both domains cholesterol exists in the lipid-bilayer-like structures. In the CCD cholesterol molecules are more ordered and sense more rigid environment than in the PCD. This difference is small and can be compared to that induced in the PCD by the ~10°C decrease of temperature. Thus, cholesterol molecules are unexpectedly dynamic in the CCD what should enhance their active interaction with the PCD. It is suggested that the EPR spin-labeling approach can discriminate the fraction of cholesterol that forms the CCD within the phospholipid bilayer from the fraction that forms the cholesterol structures outside the bilayer.

**3406-Pos Board B511**
**Discrimination and Characterization of Cholesterol Crystalline Domains using EPR Spin-Labeling: Application to Lens Lipid Membranes**
**Laxman Mainali**, Marija Raguz, Witold K. Subczynski.
Saturation-recovery EPR at X-band (9.4 GHz) and W-band (94 GHz) was used to discriminate cholesterol crystalline domains (CCDs) in model phospholipid membranes and membranes made of pigs lens lipids. We used dual-probe saturation-recovery EPR approach with cholesterol analogue spin labels, CSL or ASL, and oxygen (hydrophobic) or NiEDDA (polar) relaxation agents. The CCD was present in all of the phospholipid membranes when the cholesterol-to-phospholipid mixing ratio exceeded the cholesterol solubility threshold. Saturation-recovery curves show that spin labels detect a single homogenous environment in membranes made from cortical lipids. However, in membranes made from nuclear lipids, two domains were detected and were assigned to the bulk phospholipid-cholesterol domain and the CCD, respectively. Profiles of the oxygen transport parameter (oxygen diffusion-concentration product) across these domains contain significant structural information. These profiles also allow calculating the oxygen permeability coefficient across domain. The evaluated upper limit of the oxygen permeability coefficient across the CCD at 37°C (34.4 cm/s) is significantly lower than across the water layer of the same thickness (85.9 cm/s), indicating that the CCD can form a barrier for oxygen transport in the lens nucleus.
The new capabilities in measurements of the oxygen transport parameter at W-band are demonstrated and compared with results obtained at X-band. The loop-gap resonators used for W-band measurements have a sample volume of 30 nL, while the sample volume of the loop-gap resonator used for X-band is 3μL. These specifications ensure feasibility of experiments planned for samples with a limited sample volume. For example, the oxygen transport parameter profiles measured at X-band for lens lipid membranes were obtained for samples prepared from 50 - 100 eyes. Reliable profiles at W-band can be obtained based on samples prepared from one eye.

**3407-Pos Board B512**
**Cholesterol Transport in Model Lipid Membranes**
**Sumit Garg**, Lionel Porcar, Paul Butler, Francisco Castro-Roman, Ursula Perez-Salas.
Proper intracellular cholesterol transport is required for a healthy functioning of the cell and any disorder can lead to diseases, ranging from neurodegenerative Niemann Pick TYPE-C and Alzheimer, to cholelithiasis and atherosclerosis. However, the progress in the area of intra-cellular cholesterol transport is hampered by the huge inconsistencies in the reported value of cholesterol transport rates probably linked to the requirement of cholesterol tag or extraneous compounds such as cyclodextrine for such measurements. We recently reported a novel approach which employs Small Angle Neutron Scattering as a tool for in situ measurement of the cholesterol intra and inter-membrane exchange rates without recourse of cholesterol tags or extraneous compounds. Interestingly we found that cholesterol inter-membrane flipping is rather slow with half-life of a few hours. These results are in odds with the widely accepted belief that the presence of small hydrophilic group on cholesterol head should allow cholesterol to flip quickly through the hydrophobic acyl chain region of lipid membrane. In addition, we show that replacing cholesterol with the relatively benign dehydroergosterol or simply adding 2mM of cyclodextrine significantly affects and accelerates cholesterol flipping rates. To further develop the biophysical

mechanism for cholesterol trans-membrane flipping, transfer rates were measured for few cholesterol analogues with a slightly different molecular structure and thereby the geometrical and chemical compatibility with lipids. In addition, MD simulations were performed to measure the energetic and get the better understanding at molecular level for cholesterol transport. Overall, this work provides new insight in to cholesterol transport behavior in model lipid membrane.

**3408-Pos Board B513**

**Unique Cholesterol Transport Behavior in Phosphoserine Vesicles: A Small Angle Neutron Scattering Study**

**Sumit Garg**, Lionel Porcar, Paul Butler, Ursula Perez-Salas.

Cholesterol is critical for various cellular functions; however its excess is toxic. Cholesterol levels are maintained by various cholesterol metabolic pathways which depend critically on Intracellular cholesterol transport. Cholesterol is not homogeneously distributed in cell with 60-70% of cellular cholesterol present in plasma membrane and only 0.01-0.5% of cellular cholesterol present in endoplasmic reticulum. Any disruption in cholesterol distribution disorder has been linked to diseases such as Niemann Pick TYPE-C and Alziemers. It has been suggested that variable affinity of cholesterol for different lipids compositions could be one of the possible reason for the uneven distribution of cholesterol within cell. Traditionally the cyclodextrine or cholesterol oxidases have been used to measure the relative affinity of cholesterol for a particular lipid composition. However, the possible disruption of lipid-cholesterol interactions due to these molecules is unknown. This present study employs small angle neutron scattering to measure the cholesterol inter- and intra-membrane transport rate in model lipid vesicle without employing cholesterol tags and molecules such as cyclodextrine or cholesterol oxidase. The diffusion behavior was compared between two POPC vesicles and two POPS vesicles. Interestingly cholesterol exchange kinetics follows a non-continuous Arrhenius behavior in POPS membranes as compare to linear behavior in POPC membranes. Further cholesterol exchange kinetics was compared from POPC to POPS vesicles and POPS to POPC vesicles. Interestingly equilibrium cholesterol distribution changes with both cholesterol concentration and temperature. To our surprise we found that even a small amount of cyclodextrine can significantly shift the equilibrium distribution of cholesterol between POPS and POPC vesicles. Overall this wok provides insight in to POPS-cholesterol interaction and potential role of POPS is regulating intracellular cholesterol transport.

**3409-Pos Board B514**

**Fluorogenic-Antioxidants: Novel Probes for Visualizing Reactive Oxygen Species in the Lipid Membranes of Live Cells**

Katerina Krumova, **Gonzalo Cosa**.

We are pioneering the preparation of lipophilic fluorogenic antioxidant probes for the specific imaging of ROS in the membrane of live cells. Our strategy involves synthesizing a two segment receptor-reporter type free radical scavenger-fluorophore probe (an off-on fluorescent antioxidant indicator). The receptor segment in the probe mimics the structure and activity of the naturally occurring antioxidant $\alpha$-tocopherol. A covalently tethered bodipy fluorophore serves the purpose of reporting, via emission enhancement, structural changes at the receptor end which result from the radical scavenging activity of the receptor.

Here we will present our most recent results involving the preparation of a second generation set of probes relying on newly synthesized bodipy dyes with improved redox properties. The new fluorogenic antioxidant probes undergo a 30 fold fluorescence enhancement upon reaction with peroxyl radicals in model lipid membranes. We will also illustrate a high-throughput fluorescence method enabled by the new probes, for the rapid determination of relative rates of free radical scavenging by $\alpha$-tocopherol analogues. Rates are evaluated for tocopherol analogues with a modified lipophilic tail, when embedded in liposomes prepared from either unsaturated or saturated lipids, upon exposure to either hydrophilic or lipophilic peroxyl radicals. This work provides new insights and a quantitative understanding on the critical role of lipid diversity in modulating chemical reactions in the lipid milieu. Finally studies will be described where we utilize these new probes to image ROS in the lipid membrane of live cells.

**3410-Pos Board B515**

**Relationships Between Bilayer Phase and Equilibration Rates of Patman and Laurdan**

**Hannabeth A. Franchino**, John D. Bell.

Patman is a fluorescent membrane probe related to laurdan. The structural distinctions between the two probes are the lengths of the aliphatic tails (eleven carbons in laurdan and fifteen in patman) and the presence of a trimethylammonium forming a positively-charged head on patman. Studies exploring patman as a probe to detect membrane properties during apoptosis revealed that the two edges of the emission spectrum (435 and 500 nm) stabilize at different rates as the probe binds to the cell membrane. To test whether these differences represent dissimilarities in probe binding to ordered and disordered domains, experiments were conducted to monitor patman equilibration with bilayers composed of various mixtures of saturated and unsaturated phosphatidylcholines at temperatures above, at, and below the main thermotropic phase transition. In general, patman equilibrated more rapidly with bilayers in the liquid disordered phase than in the solid ordered phase. With solid phase membranes, the fluorescence stabilized faster at 435 nm than at 500 nm. Similar yet more subtle results occurred in the lipid disordered phase. In contrast, the situation was reversed at the phase transition temperature; equilibration was faster at 500 nm than at 435 nm. To determine whether these results reflected specific properties of patman, the experiments were repeated with laurdan, and several distinctions were observed. First, equilibration with solid phase lipids was faster than for patman and not different from equilibration with the fluid phase. Second, differences in rates between the two wavelengths were less than with patman for solid phase membranes but greater than with patman for melted bilayers. Third, at the phase transition temperature, equilibration rates favored 435 nm over 500 nm, the opposite of the result obtained with patman. Computer simulations were used to assist with interpretation of these results.

**3411-Pos Board B516**

**Fluorescent Correlation Spectroscopy and Raster Image Correlation Spectroscopy as a Tool to Measure Diffusion in the Human Epidermis**

**Jonathan Brewer**, Maria Bloksgaard, Jakub Kubiak, Luis Bagatolli.

Structural and dynamical characterization of skin tissue is vital for understanding the behavior of healthy and diseased skin tissue. Our objective is to develop protocols to measure the local diffusion of substances with different physical properties (for example amphiphilic or hydrophilic drugs) in the epidermis and dermis using fluorescent lipid analogs and hydrophobic dyes as model systems. As an example we use fluorescent labeled liposomes with a lipophilic dye in the bilayer and a hydrophilic dye inside. Using two color FCS (Fluorescent Correlation Spectroscopy) and two color RICS (Raster image correlation spectroscopy) we determine the diffusion and if intact liposomes penetrate the epidermis or if the burst before penetration. The experiments were preformed on a custom build multi-photon microscope[1]. Finally advantages and disadvantages of the different techniques for measuring diffusion in skin tissue are compared and discussed.

[1] J. Brewer, J. Bernardino de la Serna, K. Wagner and L.A. Bagatolli. Multiphoton Excitation Fluorescence Microscopy in planar membrane systems , Biochim Biophys Acta,doi:10.1016/ j.bbamem.2010.02.024 (2010).

[2] Susana A Sánchez and Enrico Gratton. Lipid-protein interactions revealed by two-photon microscopy and fluorescence correlation spectroscopy. Acc Chem Res 38(6): 469-77, 2005.

[3] Qiaoqiao Ruan, Melanie A Cheng,Moshe Levi, Enrico Gratton, and William W Mantulin. Spatial-temporal studies of membrane dynamics: scanning fluorescence correlation spectroscopy (SFCS). Biophys J 87(2): 1260-7, 2004.

[4] Claire M Brown, Rooshin B Dalal, B Hebert, Michelle A Digman, AlanR Horwitz, and Enrico Gratton. Raster image correlation spectroscopy (RICS) for measuring fast protein dynamics and concentrations with a commercial laser scanning confocal microscope. J Microsc 229(1): 78-91, 2008.

**3412-Pos Board B517**

**Concentration Dependent Membrane Anchor Colocalization Study by Fluorescence Cross-Correlation Spectroscopy in Live Cells**

**Sara B. Triffo**, Hector H. Huang, Adam W. Smith, Jay T. Groves.

Membrane anchors such as protein lipidations and glypiations have been proposed to play essential roles in the sorting and organization of plasma membrane-associated proteins, especially those involved in cell signaling. Here, we investigate the concentration dependence and variability of anchor colocalization in live cells by transfecting various cell types with pairs of fusion proteins created by replacing all but short tails of natively lipidated proteins with either red or green fluorescent proteins. These fusion proteins remove any native protein-protein interactions while fluorescently tagging membrane anchors in live cells. To observe sub-cellular organization, we use Fluorescence Cross-Correlation Spectroscopy (FCCS) to quantify the dynamic colocalization between green- and red-labeled anchors. FCCS allows observations of dynamic colocalization in live cells at a greater range of separation distances than is allowed by FRET, and because it is a dynamic measurement FCCS avoids ambiguous or false positive colocalization that can result from static studies. Fusion protein expression level, as determined by overall intensity of cell fluorescence, naturally varies in a population of transiently transfected cells. Using this to our advantage, we are able to observe cells within a wide range of protein expression and explore trends between concentration and fusion protein colocalization. We also analyze variation in the amount of colocalization and observe a difference between the variability from cell-to-cell and the variability from spot-to-spot within one cell across several anchor types and different cell lines.